

**LISTING OF THE CLAIMS**

This listing of claims replaces all prior listings.

1. (CURRENTLY AMENDED) A phenol-containing mono-phase composition for isolating purified RNA comprising phenol at a final concentration ranging from about 3%<sup>w/w</sup> to about 98%<sup>w/w</sup> and a buffer sufficient to maintain a pH of the final composition in the range from about pH 3.6 to below pH 4.0.
2. (CURRENTLY AMENDED) A phenol-containing mono-phase composition for isolating purified RNA comprising phenol at a final concentration ranging from about 3%<sup>w/w</sup> to less than 30%<sup>w/w</sup>, and a buffer sufficient to maintain a pH of the final composition in the range from about pH 3.9 to about pH 5.5.
3. (ORIGINAL) The composition according to claims 1 or 2 where the buffer is selected from at least one of acetate, citrate, phosphate, phthalate, tartrate, lactate, or mixtures thereof.
4. (ORIGINAL) The composition according to claims 1 or 2 further comprising at least one ribonuclease inhibitor.
5. (ORIGINAL) The composition of claim 4 wherein the ribonuclease inhibitor is selected from at least one of proteinase K, ribonuclease inhibitor from human placenta, vanadyl ribonucleoside complex, chaotropic salts, or mixtures thereof.

6. (ORIGINAL) The composition of claim 5 wherein the chaotropic salts are selected from at least one of urea salts, guanidine salts, or mixtures thereof.
7. (ORIGINAL) The composition of claim 6 wherein the guanidine salts are selected from at least one of guanidine thiocyanate or guanidine hydrochloride at a final concentration in the range of about 0.5 M to about 6 M.
8. (ORIGINAL) The composition according to claims 1 or 2 further comprising a detergent.
9. (ORIGINAL) The composition of claim 8 wherein the detergent is selected from at least one of sarcosine, polyoxyethylenesorbitan, a dodecylsulfate salt, or mixtures thereof.
10. (ORIGINAL) The composition according to claims 1 or 2 further comprising an inorganic or organic salt and a chelating agent.
11. (ORIGINAL) The composition of claim 10 wherein the inorganic or organic salt is selected from at least one of chlorides, phosphates, bromates, acetates, citrates, phthalates, tartrates, lactates, or thiocyanates of sodium, potassium, lithium or ammonium.
12. (ORIGINAL) The composition of claim 10 wherein the chelating agent is selected from at least one of citrates, ethylenediamine tetraacetic salts, or mixtures thereof.

13. (ORIGINAL) The composition according to claims 1 or 2 further comprising phenol derivatives selected from at least one of phenylethanol, propylene phenoxylol, thymol, butylphenol, or mixtures thereof at a final concentration up to about 5%<sup>w/w</sup>.

14. (ORIGINAL) The composition according to claims 1 or 2 further comprising phenol solubilizers selected from at least one of polyalcohols, monoalcohols, and guanidine salts.

15. (ORIGINAL) The composition of claim 1 further comprising at least one organic compound in a concentration ranging from about 1%<sup>w/w</sup> to about 5%<sup>w/w</sup> sufficient to increase the density of the composition.

16. (ORIGINAL) The composition of claim 15 wherein the organic compound is selected from at least one of cyclohexyl bromide, dibromopropane, dichlorobenzoic acid, and mixtures thereof.

17-25. (CANCELED)

26. (ORIGINAL) A phenol-free phase separation composition for use in isolating purified RNA by phase separation comprising at least one hydrophobic organic solvent at a final concentration in the range from about 10%<sup>w/w</sup> to about 40%<sup>w/w</sup>, and at least one acid sufficient to maintain a pH in the range of about pH 3.6 to below pH 4.0 during phase separation, and an optional acid solubilizer.

27. (ORIGINAL) The composition of claim 26 wherein the organic solvent is at least one of chloroform, carbon tetrachloride, bromochloropropane, bromonaphtalene, or bromoanisole.

28. (ORIGINAL) The composition of claim 26 wherein the acid is at least one of formic acid, acetic acid, trichloroacetic acid, aminocaproic acid, lactic acid, or chlorophenylacetic acid.

29. (ORIGINAL) A method for isolating purified RNA from a biological sample comprising

- a) treating the sample with a reagent comprising phenol at a final concentration ranging from about 10%<sup>w/w</sup> to about 60%<sup>w/w</sup> and at least one ribonuclease inhibitor,
- b) mixing the sample with at least one hydrophobic solvent while maintaining a pH in the range from about pH 3.6 to below pH 4.0,
- c) recovering purified RNA from an aqueous phase to which about an equal volume of a water-soluble organic solvent is added to precipitate the purified RNA, and
- d) washing and solubilizing the precipitated RNA.

30. (ORIGINAL) The method of claim 29 wherein the reagent in (a) further comprises a buffer selected from at least one of acetate, citrate, phosphate, phthalate, tartrate, lactate, or mixtures thereof.

31. (ORIGINAL) The method of claim 29 wherein the reagent in (a) further comprises at least one ribonuclease inhibitor.
32. (ORIGINAL) The method of claim 31 wherein the ribonuclease inhibitor is selected from at least one of proteinase K, ribonuclease inhibitor from human placenta, vanadyl ribonucleoside complex, chaotropic salts, or mixtures thereof.
33. (ORIGINAL) The method of claim 32 wherein the chaotropic salts are selected from at least one of urea salts, guanidine salts, or mixtures thereof.
34. (ORIGINAL) The method of claim 33 wherein the guanidine salts are selected from at least one of guanidine thiocyanate or guanidine hydrochloride at a final concentration in the range of about 0.5 M to about 6 M.
35. (ORIGINAL) The method of claim 29 wherein the reagent in (a) further comprises a detergent.
36. (ORIGINAL) The method of claim 35 wherein the detergent is selected from at least one of sarcosine, polyoxyethylenesorbitan, a dodecylsulfate salt, or mixtures thereof.
37. (ORIGINAL) The method of claim 29 wherein the reagent in (a) further comprises an inorganic or organic salt and a chelating agent.

38. (ORIGINAL) The method of claim 37 wherein the inorganic or organic salt is selected from at least one of chlorides, phosphates, bromates, acetates, citrates, phthalates, tartrates, lactates, or thiocyanates of sodium, potassium, lithium or ammonium.

39. (ORIGINAL) The method of claim 37 wherein the chelating agent is selected from at least one of citrates, ethylenediamine tetraacetic salts, or mixtures thereof.

40. (ORIGINAL) The method of claim 29 wherein the reagent in (a) further comprises phenol derivatives selected from at least one of phenylethanol, propylene phenoxylol, thymol, butylphenol, or mixtures thereof at a final concentration up to about 5%<sup>ww</sup>.

41. (ORIGINAL) The method of claim 29 wherein the reagent in (a) further comprises phenol solubilizers selected from at least one of polyalcohols, monoalcohols, and guanidine salts.

42. (ORIGINAL) The method of claim 29 wherein the reagent in (a) further comprises at least one organic compound in a concentration ranging from about 1%<sup>ww</sup> to about 5%<sup>ww</sup> sufficient to increase the density of the composition.

43. (ORIGINAL) The method of claim 42 wherein the organic compound is selected from at least one of cyclohexyl bromide, dibromopropane, dichlorobenzoic acid, and mixtures thereof.

44. (ORIGINAL) The method of claim 29 wherein the sample is first treated with the phenol-free composition of either of claims 17 or 26 before step (a).

45. (ORIGINAL) The method according to claims 29 or 41 wherein the reagent in (a) is buffered to maintain a pH in the range from about pH 3.6 to below pH 4.0.

46. (ORIGINAL) The method according to claims 29 or 44 wherein step (a) is performed at a pH ranging from about pH 3.9 to about pH 9.0, and the sample is then adjusted to a pH ranging from about pH 3.6 to below pH 4.0.

47. (CURRENTLY AMENDED) [[A]] An acidic phenol precipitation method for isolating purified RNA from a biological sample comprising the steps of

- a) treating the sample with a mono-phase reagent comprising phenol at a final concentration ranging from about 3%<sup>w/w</sup> to less than 30%<sup>w/w</sup> and a buffer sufficient to maintain a pH of the composition in the range from about pH 3.6 to about pH 5.5,
- b) sedimenting or filtering the sample to obtain a purified sample substantially free of DNA, proteins, and cellular components,
- c) adding to the purified sample about an equal volume of a water-soluble organic solvent to precipitate purified RNA,
- d) sedimenting or filtering the precipitated RNA, and
- e) washing and solubilizing the precipitated RNA.

48. (CURRENTLY AMENDED) A two-step method for isolating purified RNA from a biological sample comprising

- a) treating the sample with a mono-phase reagent comprising phenol at a final concentration ranging from about 3%<sup>w/w</sup> to less than 30%<sup>w/w</sup>, at least one chaotrope, and a buffer sufficient to maintain a pH of the composition in the range from about pH 3.6 to about pH 5.5,
- b) sedimenting or filtering the sample to obtain a purified sample substantially free of DNA, proteins, and cellular components,
- c) adding to the purified sample at least one hydrophobic organic solvent and a buffer in a concentration sufficient to maintain a pH of the purified sample in the range from about pH 3.6 to below pH 4.0,
- d) recovering purified RNA from an aqueous phase to which about an equal volume of a water soluble organic solvent is added to precipitate purified RNA,
- e) sedimenting or filtrating the precipitated RNA, and
- f) washing and solubilizing the precipitated RNA.

49. (ORIGINAL) The method of claim 48 where the hydrophobic organic solvent is sufficiently dense to separate the organic phase during phase separation.

50. (ORIGINAL) The method according to claims 47 or 48 wherein the hydrophobic organic solvent is selected from at least one of caprolactone, ethylene glycol diacetate, polyethylene glycol dibenzoate, chloroform, carbon tetrachloride, bromochloropropane, bromonaphtalene, bromoanisole, or mixtures thereof.

51. (ORIGINAL) The method according to claims 47 or 48 wherein the sample is treated with the composition of (a) at about 1.5X to about 2.5X concentration, and the resulting sample is diluted to approach the non-concentrated solution.

52. (ORIGINAL) The method according to any of claims 29, 44, 47, or 48 wherein the solvent added to precipitate RNA is at least one of lower alcohols, polyalcohols, acetone, ethyleneglycol diacetate, methyl sulfoxide, or mixtures thereof.

53. (ORIGINAL) A method for isolating purified RNA from an RNA- and salt-containing solution comprising adjusting a pH of the solution with a buffer in an amount sufficient to result in a maximum pH of 3.3, thereafter precipitating the purified RNA.

54. (ORIGINAL) The method of claim 53 wherein the pH ranges from about pH 3.0 to about pH 2.7.

55. (ORIGINAL) The method of claim 53 wherein the buffer is at least one of an organic acid or an inorganic acid.

56. (ORIGINAL) The method of claim 55 wherein the acid is selected from at least one of hydrochloric acid, phosphoric acid, acetic acid, lactic acid, or mixtures thereof.

57. (ORIGINAL) The method of claim 53 wherein the salt is selected from the group consisting of sodium, potassium, lithium and guanidine salts.

58. (ORIGINAL) The method of claim 53 wherein the solution contains phenol at a concentration from about 1%<sup>w/w</sup> to about 60%<sup>w/w</sup>.

59. (ORIGINAL) A method for selectively precipitating higher molecular weight RNA from a biological sample comprising  
treating the sample with an aqueous composition comprising phenol at a final concentration ranging from about 1%<sup>w/w</sup> to about 60%<sup>w/w</sup>, at least one chaotrope, a buffer in a concentration sufficient to maintain a pH of the composition in the range from about pH 2.0 to about pH 9.0, at least one water-soluble organic solvent at a concentration from about 10%<sup>w/w</sup> to about 40%<sup>w/w</sup> to selectively precipitate higher molecular weight RNA from the sample, and  
precipitating purified higher molecular weight RNA from the sample.

60. (ORIGINAL) The method of claim 59 further comprising the step of thereafter adding additional organic solvent sufficient to increase the concentration of organic solvent to at least 50%<sup>w/w</sup> to precipitate lower molecular weight RNA, and precipitating purified lower molecular weight RNA from the sample.

61. (ORIGINAL) The method of claim 59 comprising preparing the biological sample according to any of claims 29, 44, 47, or 48 to obtain an aqueous solution of RNA, and precipitating RNA from the aqueous solution.